myosin molecules are brought together and localized to the actin bundles in the stereocilia participating in the lateral connections along with the interacting proteins such as vezatin and cadherin.
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3768-Pos
A Comparison of Mechanical Properties of Drosophila and Mouse Myosin 7a
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Myosin 7a is an unconventional myosin present in a range of organisms, and is essential in the function of sensory cells. In *Drosophila*, myosin 7a (D-M7a) is required to maintain bristle structure in Johnston's organ (the auditory center in *Drosophila*). Equivalently, in mice the absence of myosin 7a (M-M7a) disrupts stereocillia structure which adversely effects vestibular function. D-M7a and M-M7a share good sequence homology. Both have a 5-IQ lever-arm, followed by a single α-helix (SAH) domain, and an SH3 domain separating two MyTH4-FERM domains. Here we use the three-bead optical trap assay to compare the kinetics and mechanics of myosin 7a from insect (*Drosophila*) and vertebrate (mouse) species. We use a truncated D-M7a construct (D-M7aTD1), cropped after the SH3 domain to prevent auto-regulation. Due to difficulties with M-M7a expression, a shorter construct truncated after the SAH was used (M-M7aSAH). Data were taken at 50mM ionic strength with 10μM ATP. Step sizes of 10 and 18nm were observed for the D-M7aTD1 and M-M7aSAH, respectively. Variations in light chain binding, and geometric hindrances resulting from the shorter tail in M-M7aSAH, may contribute to this difference. The dwell time data from D-M7aTD1 were fitted well by a single exponential, giving an actin detachment rate (K_{det}) of $1.1s^{-1}$. This compares favourably with the biochemically determined ADP release step. Interpretation of the dwell time data from M-M7aSAH is less straight forward. The data is poorly fit by a single exponential. A double exponential gives fast ($9.5s^{-1}$) and slow ($0.8s^{-1}$) K_{det} rates. Comparison to biochemical data suggests the fast rate is related to ADP release, whereas the slow rate may represent ATPase cycling. We conclude *Drosophila* and mouse myosin 7a exhibit generally similar mechanical properties, though appear differently tuned, perhaps for their species specific function.

3769-Pos
Kinetic Analysis Reveals Differences in the Binding Mechanism of Calmodulin and Calmodulin-Like Protein to the 3 IQ Motifs in Myosin-10
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Myosin-10 (Myo10) is an unconventional myosin associated with dynamic actin remodeling implicated in a multitude of cellular functions. Like most unconventional myosins, Myo10 binds calmodulin (CaM) as the principal light chain. In epithelial cells Myo10 also binds calmodulin-like protein (CLP) as a tissue-specific light chain, resulting in increased Myo10 levels and Myo10-dependent cell motility. This raises questions as to how CaM and CLP compete for the individual IQ sites on Myo10. Indeed, there is little information on the kinetics of light chain binding to any unconventional myosin. Moreover, how Ca^{2+} affects the binding of CaM and CLP to the IQ motifs in Myo10 is unknown. We performed equilibrium and fast-kinetic experiments to elucidate the mechanism of binding of both CaM and CLP to each of the three IQ motifs in Myo10. Our results show that while CaM and CLP bind with moderate affinity to the isolated IQ2 domain in the absence of Ca^{2+}, both light chains display dramatically increased affinity for each of the three IQ domains in the presence of Ca^{2+}. The studies further indicate different binding mechanisms for CLP and CaM to IQ3, suggesting structural differences between the CaM-IQ3 and CLP-IQ3 complexes and supporting differential effects of the two light chains on Myo10 regulation and stability.

3770-Pos
The Molecular Adaptations of Myosin X for Bundled Actin Filament Tracks
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To properly self-organize, cells must be able to direct cargo to specific locations. Although many molecular motors are known to drive cargo transport, the address system that these motors use to move to the proper destination is poorly understood. Recently, we showed that myosin X has a preference for bundled actin filaments. This preference for bundles allows myosin X to identify filopodia, a limited population of actin filaments within the cell. To clarify the bundle selection mechanism, we performed single-molecule mechanical measurements to determine the stepping pattern on bundles. Our observed ~18 nm stepsize is consistent with a filament-straddling mechanism, where each head of myosin X binds to a unique filament in the bundle. A dissection of the domains required for bundle selection reveals a surprising role for the myosin X tail, a region which is likely far from the bundle itself. We find that targeted insertion of a glycine-rich flexible linker within the tail abolishes bundle selectivity, suggesting that the tail adopts a rigid structure that is essential for identifying bundles.

3771-Pos
A Structured Domain is Responsible for Bundle Selection of Myosin X
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Cells organize their contents and regulate cell shape and mechanics through molecular motors functioning on cytoskeletal filaments. Presented with many apparently similar tracks within the cortex, how myosins identify the few actin filaments that lead to their correct cellular destinations is largely unknown. Myosin X, an actin-based motor that concentrates at the distal tips of filopodia, selects the fascin-actin bundle at the filopodial core for motility. While poorly processive on single actin filaments, it takes processive runs on actin bundled by fascin. Such a bundle is the precise structure to which myosin X localizes in vivo. Using single molecule optical trapping experiments we determined the step size to be 17 nm, half of the 36 nm pseudo-helical actin repeat essential for motors to be processive on single actin filaments. This suggested that straddling two filaments within a bundle stimulates this motor's processivity. Using combinatorial chimeric constructs of myosin V and myosin X, we show that the post-IQ region, not the short lever arm (three IQ repeats) or the motor domain, is the main contributor to myosin X's selectivity. This region contains two structures of interest: a charged single alpha-helix (SAH), which may impart unique mechanical or affinity properties to the motor, and a coiled-coil dimerization motif. The structural character of this region was perturbed by insertion of free swivels either before or after the SAH domain. The post-SAH swivel mutant showed no preference for bundled actin for motility, thus providing support to a selectivity model where the search-space of the forward head for the next binding site is constrained to neighboring filaments in a bundle. This result provides insight into the ability of nature to fine-tune myosin motors to serve their specific functions in the cell.

3772-Pos
A Receptor Mediated Delivery System for Single Molecule Imaging in Live Cells
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The high complexity of the eukaryotic cytoskeleton arises from many proteins displaying multiple functions. Live, intact cells are an indispensable system for investigating motility of molecular motors, which depend on their intracellular environments. Here we present a novel system of delivering myosin motors into the cell to investigate their motility by single molecule imaging on the native cytoskeleton. This technology utilizes receptor mediated delivery (RMD) of fluorescently labeled motors. A conjugate of the desired myosin motor and substance P (SP) is internalized upon interaction with the neurokinin-1 receptor (NK-1), allowing the myosin motors access the cell without compromising the integrity of the membrane. RMD has no harmful impact on the cell, leaving the cell membrane intact and the sensitive structures preserved.
We previously demonstrated that myosin X selects the fascin-actin bundle at the filopodial core for motility. Here we show that this motor is successfully delivered with RMD, undergoes endosomal escape and finds its way to its native working environment simply by its functional preference for these unique structures. This is significant as it not only confirms that myosin X recognizes the local structural arrangement of filaments, but also further indicates that the details of cellular actin organization do impact the activity of unconventional myosins. Information on myosin motor motility obtained by RMD allows for the construction of a road map of the actin structures and enables a comparison between various cell types. These paths reveal both the spatial arrangement of the actin filaments (reflecting the complexity and density of cytoskeletal meshwork) as well as the individual motility preferences of myosin motors across cell types.

3773-Pos
The Dynamics for Myosin-X Induced Filopodia Protrusion
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Filopodia are actin-rich finger-like cytoplasmic projections extending from the leading edge of cells. Unconventional myosin-X is involved in the protrusion of